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urcl.*

34. The method of claim 33, wherein said endonuclease recognition site is selected from the group consisting of Class I I-endonuclease sites, Class II I-endonuclease sites, Class III I-endonuclease sites, Class IV I-endonuclease sites, and Class V I-endonuclease sites.

35. The method of claim 34, wherein said endonuclease recognition site is a Class I I-endonuclease site.

36. The method of claim 35, wherein said endonuclease recognition site is selected from the group consisting of I-SceI, I-SceIV, I-Csml, and I-Panl sites.

37. The method of claim 36, wherein said endonuclease recognition site is an I-SceI site. --

add c27

REMARKS

Entry and consideration of this amendment is respectfully requested.

Claims 1-26 have been canceled. New claims 27-37 find support throughout the specification, for example on pages 20-21 and Fig.6. Accordingly, no new matter is entered by amendment.

Applicants submit herewith a Sequence Listing and have amended the specification to conform with the requirements of 37 C.F.R. §§ 1.821-1.825.

Applicants request the use of the computer-readable form of the Sequence Listing in U.S. Application Serial No. 08/417,226, filed April 5, 1995, now U.S. Patent No. 5,962,327, issued October 5, 1999.

I hereby state that the contents of the paper copy of the Sequence Listing in this application and the computer-readable form of the Sequence Listing in U.S. Application Serial No. 08/417,226, filed April 5, 1995, submitted in accordance with 37 C.F.R. § 1.821(c) and (e), respectively, are the same.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with Markings to Show Changes Made."

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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Dated: April 18, 2001

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Version with Markings to Show Changes Made

In the Specification:

The paragraph beginning on line 4 of page 5 has been amended as follows:

Accordingly, this invention aids in fulfilling these needs in the art. Specifically, this invention relates to an isolated DNA encoding the enzyme I-SceI. The DNA has the following nucleotide sequence:

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On page 7 beginning at line 2, paragraphs 1-11 have been amended as follows:

This invention will be more fully described with reference to the drawings in which:

Fig. 1 depicts the universal code equivalent of the mitochondrial I-Scel gene (SEQ ID NO:1).

Fig. 2 depicts the nucleotide sequence of the invention encoding the enzyme I-Scel and the amino acid sequence of the natural I-Scel enzyme (SEQ ID NOS: 5 and 2).

Fig. 3 depicts the I-Scel recognition sequence and indicates the possible base mutations in the recognition site and the effect of such mutations on stringency of recognition (SEQ ID NOS: 6, 7, and 8).

Fig. 4 is the nucleotide sequence and deduced amino acid sequence of a region of plasmid pSCM525. The nucleotide sequence of the invention encoding the enzyme I-Scel is enclosed in the box (SEQ ID NOS: 9 through 16).

Fig. 5 depicts variations around the amino acid sequence of the enzyme I-Scel (SEQ ID NO: 2).

Fig. 6 shows Group I intron encoding endonucleases and related endonucleases (SEQ ID NOS: 17-44).

Fig. 7 depicts yeast expression vectors containing the synthetic gene for I-Scel.

Fig. 8 depicts the mammalian expression vector PRSV I-Scel.

Fig. 9 is a restriction map of the plasmid pAF100. (See also YEAST, 6:521-534, 1990, which is relied upon and incorporated by reference herein).

Figs. 10A and 10B show the nucleotide sequence and restriction sites of regions of the plasmid pAF100 (SEQ ID NOS: 45-50).

On page 12, the last paragraph has been amended as follows:

The enzyme I-Scel has a known recognition site. (ref. 14.) The recognition site of I-Scel is a non-symmetrical sequence that extends over 18 bp as determined by systematic mutational analysis. The sequence reads: (arrows indicate cuts)

5' TAGGGATAACAGGGTAAT 3' (SEQ ID NO:51)
3' ATCCCTATTGTCCCCATTA 5' (SEQ ID NO:52).

↑

On pages 41 to 42, the bridging paragraph has been amended as follows:

-e- The supernatant of this clone was used to infect other mouse cells (1009) by spreading 10^5 virus particles on 10^5 cells in DMEM medium with 10% fetal calf serum and 5 mg/ml of "[polybrain] polybrene (hexadimethrine bromide)". Medium was replaced 6 hours after infection by the same fresh medium.

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